The role of Ku at the telomere

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I. Introduction

Ku, originally identified as the autoantigen associated with systemic lupus erythematosus (1), is an abundant nuclear protein with fascinating multi-functionality. Ku exists primarily in a heterodimer form composed of ~70 and ~86 Kd subunits (referred to as Ku70 and Ku80), which can also form into a larger functional complex termed the DNA-dependent protein kinase (DNA-PK). In addition to Ku, the DNA-PK complex contains the 470 Kd DNA-dependent protein kinase catalytic subunit (DNA-PKcs) (2, 3).

Surprisingly, Ku along with several other proteins that play critical roles in double-strand break repair, through the non-homologous end joining (NHEJ) pathway or during site-specific recombination of V(D)J gene segments, have recently been shown to play additional roles in capping telomeres or preventing chromosome end fusions (4-10). Therefore, Ku can function in seemingly opposite ways, joining DNA ends as opposed to preventing telomere fusions, depending on its nuclear context or microenvironment. Ku has also been reported to play critical roles during transcriptional regulation and the modulation of chromatin structure (11-17).

In this chapter, we will focus primarily on the role of Ku at the telomere. Our current understanding of the role Ku plays at the telomere has come mainly from yeast and mammalian studies. Ku has been shown to function in telomere capping (or preventing telomere end fusions), telomeric DNA length control, and telomere silencing. Ku is known to interact with certain telomere proteins in both yeast and mammals (see details below), though the exact role that Ku plays at the telomere and how it localizes to the telomere is not presently known. Importantly, despite the well-known binding affinity of Ku for DNA termini, there is currently no evidence that Ku binds directly to the ends of telomeric DNA through a sequence independent manner. Furthermore, mammalian Ku does not bind directly to internal regions of telomeric DNA in a sequence dependent manner (8). It is likely that the telomere is a dynamic structure with various telomere components forming many different intermolecular interactions depending on the cell cycle, age of the organism and other biological conditions. Within this chapter, we will explore possible roles for Ku in telomere maintenance and several possible intermolecular contacts that may facilitate its function.

Before focusing on the telomere, we will provide a brief description of Ku proteins intermolecular contacts during other important cellular processes. These background sections are not intended to provide the reader with a complete review of Ku, but are intended to inform the reader of the capabilities and potentials of Ku, and provide an understanding of how Ku might function at the telomere. For a more comprehensive summary of Ku, the reader is referred to several excellent reviews (3, 18). Remarkably, three basic types of intermolecular interactions have been assigned to the Ku heterodimer: 1) sequence independent DNA end binding, 2) sequence dependent DNA/RNA binding to internal regions of nucleic acids and 3) protein-

protein interactions. Understanding the basic molecular associations, which Ku forms at the telomere is required for a continued understanding of the basic actions of Ku at the telomere.

II. Ku binds DNA ends in a sequence independent manner.

Analysis of X-ray sensitive mutant rodent cell lines and mice deficient for Ku revealed its requirement for joining DNA double-strand breaks via the non-homologous end joining (NHEJ) pathway and during V(D)J recombination (15, 19-23). NHEJ functions to repair DNA double-strand breaks created throughout the genome by exogenous and endogenous DNA damaging agents. NHEJ does not require sequence homologies between the two recombining DNA ends. V(D)J recombination is the recombinational joining of gene segments to form functional immunoglobulin (Ig) and T-cell receptors (TCR) genes during the development of lymphoid cells. V(D)J recombination involves double-strand DNA cleavage between coding sequences and neighboring recombination signal sequences (RSSs). Recombination of these DNA segments after modification of their ends permits the assembly of an immense variety of immunoglobulins and T cell receptor genes. Therefore, both the NHEJ and V(D)J recombination processes involve the joining of double-strand DNA ends composed of immense DNA sequence variation (2, 16, 24).

The Ku heterodimer exhibits intriguing *in vitro* sequence independent or non-specific double-strand DNA end binding properties that most likely play an important role in the recognition and joining of cleaved non-homologous DNA ends in NHEJ and V(D)J recombination. The dissociation constant for the end binding activity of Ku is reported to be between 1.5-4.0 X 10^{-10}/M (25, 26). The Ku heterodimer binds directly to double-strand DNA ends but also binds with high affinity to single stranded nicks and gaps in a sequence independent manner (25, 27, 28). Additionally, when bound at a DNA end, Ku is capable of translocating to internal DNA positions in an energy independent manner (27, 29, 30). Biochemical studies of the DNA-PKcs and Ku components of the DSB repair pathway have revealed that the Ku heterodimer is required for the stable interaction of the DNA-PK catalytic subunit (DNA-PKcs) with DNA ends and for kinase activation (31, 32). The Ku heterodimer is believed to target DNA-PKcs to DNA double-stranded breaks *in vivo* and the resulting activation of serine/threonine kinase activity of DNA-PKcs likely initiates critical aspects of the DSB joining process in mammalian cells (2, 23). However, essentially nothing is known concerning the specific function of Ku within the cell. Understanding the role Ku protein plays *in vivo* during the joining of double-strand DNA ends is an active and important area of current research.

III. Ku binds to DNA and RNA in a sequence specific manner.

In addition to DNA sequence independent end binding, many groups have reported the binding of Ku to internal regions of DNA in a sequence dependent manner (18). Several reports show a DNA sequence specific binding activity of Ku to upstream promoter elements during the

regulation of gene expression. For example, it was reported that Ku functions to repress glucocorticoid-induced MMTV transcription by binding to negative regulatory element 1 (NRE1) (11, 33). The latter study was the first to use covalently closed micro-circles in electrophoretic mobility shift assays (EMSA) to test Ku for sequence specific binding *in vitro*. This methodology was a major advancement in the study of Ku proteins sequence dependent DNA binding, allowing Ku proteins sequence independent end binding activity to be experimentally separated from its sequence specific binding. Ku binds with nearly the same affinity to the internal NRE1 binding site (Kd=0.84 X 10^{-9}/M) as to DNA ends via its sequence independent DNA end binding activity (11). Additionally, it was reported that Ku70 binds to the enhancer region of the T-cell receptor beta-chain gene (34), and is a negative regulator of hsp70 gene expression (35, 36). Since Ku possesses a high affinity non-specific DNA end binding activity, it is absolutely essential that special experimental care be taken to determine whether any putative interaction with a specific nucleic acid sequence or structure is truly direct as opposed to a DNA-mediated or bridge-associated.

Yet another functional role for Ku involving its sequence specific DNA binding activity was identified by the discovery that Ku binds with high affinity to matrix attachment regions (MARs) contained within DNA micro-circles (13). MARs are DNA sequences located at the base of chromatin loops, which are thought to attach to the nuclear matrix (37). Base unpairing regions (BURs) are found within MARs and are thought to contribute to the formation of the chromatin loop domain structures (37). Base unpairing region affinity chromatography was used to co-purify poly(ADP-ribose) polymerase, Ku and DNA-PKcs from a nuclear cell extract (13). Therefore, Ku may play a role in the formation of loop domain structures of chromatin thereby participating in the modulation of chromatin structure.

As well as the ability of Ku to bind specifically and non-specifically to DNA, it has also been reported to bind directly to certain RNA sequences (18). One reported example is the specific association of Ku to the HIV RNA transactivation response (TAR) element (38). Additionally, using SELEX (systematic evolution of ligands by exponential enrichment) technology, an *in vitro* study was initiated to identify specific RNA sites that bind Ku with high affinity (18). Three classes of Ku-RNA binding sites were found, with most sites binding Ku with affinities less than or equal to 2 x 10^{-9}M. Recently, Ku was reported to have a possible genetic link with the yeast telomerase RNA (TLC) (39); discussed in more detail below), but whether Ku physically binds directly to the yeast telomerase RNA has yet to be determined (39). Finally, while Ku can clearly binding with high affinity to either DNA or RNA in a site-specific manner, the biological significance of these interactions requires further study.

IV. Ku forms protein-protein interactions

Ku has been reported to form many different protein-protein interactions. As discussed above, Ku forms a complex with the DNA-dependent protein kinase catalytic subunit (2). Additionally, Ku has been reported to interact with the Werner syndrome protein (WRN) (40, 41), the heterochromatin protein 1 alpha (42), the XRCC4/Ligase IV complex (43), terminal deoxynucleotidyl transferase (44), the proto-oncogene p95vav (45), poly(ADP-ribose) polymerase (13), and several other proteins (2, 46, 47). In addition, the only reported intermolecular Ku protein interactions with the telomere are through protein-protein complexes with the telomere proteins Sir4 in yeast and the telomeric DNA binding protein TRF1 in mammals (8, 48), see below for more details). It is important to reiterate that due to the high affinity non-specific end binding activity of Ku, differentiating between DNA mediated interactions and true protein-protein interactions requires special experimental care.

V. Telomeres and telomerase.

Telomeres, composed of repetitive DNA sequences bound by telomere protein complexes, function to protect the chromosome termini from fusion events and promote chromosomal end replication (49). Telomeric DNA is synthesized by telomerase, a specialized ribonucleoprotein (RNP) reverse transcriptase. Known essential core components of telomerase include the telomerase RNA (TER), containing a short template sequence that is copied into telomeric DNA, and a protein reverse transcriptase subunit (TERT) (50-52). A minimal telomeric DNA length, and in some situations an active telomerase are required for chromosome stability and cellular viability (53, 54). Telomere maintenance requires a homeostatic balance between addition of telomeric sequences by telomerase, repression of telomerase, and persistent capping activity by telomeric proteins and the formation of structures such as the t-loop (48, 49, 55). Failure to maintain telomere length or function can lead to a form of replicative senescence in ciliates, yeast and mammalian cells (50, 56-58). Most differentiated human somatic cells lack telomerase activity and activation of telomerase is characteristic of most established human cell lines and tumors (59). Correspondingly, experimental activation of telomerase allows certain virus-transformed human cell lines to bypass normal cellular replicative senescence and crises and continue proliferation (53, 58).

The well-known capacity of Ku for DNA binding, either sequence independent or dependent suggested that Ku might bind directly to telomeric DNA (Figure 2C and Figure 3C). It was initially hypothesized that Ku may bind telomeric DNA ends sequence independently and/or bind to internal regions of telomeric repeats through its reported sequence dependent mode. But at present there is no clear evidence that Ku binds directly to telomeric DNA and Ku does not specifically recognize internal regions of vertebrate telomeric DNA, $(T_2AG_3)_n$ repeats, within covalently closed micro-circular DNA (8). Indeed, it is important to consider that the function of Ku at the telomere is likely very distinct, possibly in direct opposition, from its action in joining

DNA ends, since the critical functional role of telomeres is to cap or prevent telomere end fusions (8, 10, 60, 61). Some fascinating insights into the role of Ku at the telomere have recently been discovered, which will be discussed in the remaining sections of this chapter.

VI. Ku at the yeast telomere.

Several lines of evidence have converged over the last several years strongly supporting the conclusion that the Ku heterodimer plays a critical role in yeast telomere maintenance. Initially, two groups working with *Saccharomyces cerevisiae* found that nulls for either yKu70 or yKu80 had abnormally short telomeres (4-6, 62). In order to identify components that interact with Ku, a two-hybrid screen for factors that interact with yKu70 was used and the Sir4 protein was identified (63). The Sir4 protein was known to be important for telomere silencing, a chromosomal position effect in which genes adjacent to yeast telomeres are repressed, similar to the repression associated with heterochromatin in higher eukaryotic cells (64). In agreement with the involvement of Ku in *S. cerevisiae* telomere silencing, yKu70 nulls are defective in telomere silencing (6, 65, 66). Contrastingly, recent studies have revealed that Ku plays an important role in telomere maintenance in *Schizosaccharomyces pombe* (67, 68), yet is not required for telomere silencing (69). Direct evidence that Ku is physically localized to telomeres was initially provided by *in vivo* cross-linking experiments (using chromatin immunoprecipitation or CHIP) (70). Studies over the last ten years have suggested that Rap1 and Sir4 interact directly at the telomere in yeast (48, 71). Rap1 is a multifunctional protein, which, in addition to binding yeast upstream activating sequences (UAS), binds yeast telomeric DNA directly in a sequence dependent manner and plays an important role in telomere silencing (72). Therefore, its been hypothesized that a Ku/Sir4 complex could localizes to the telomere by an interaction via Rap1. However, Ku localizes to telomeres in a Sir4 independent manner, indicating that Sir4 is not a direct protein bridge for Ku localization to the telomere (Figure 2A; (73). In addition, there is no evidence that Ku and Rap1 interact directly. CHIP experiments demonstrated that Ku is in close proximity to the telomere, but whether Ku binds directly to yeast telomeric DNA can not be determine by this method, so the possibility clearly exists that yKu localizes to the telomere via a telomere protein component (Figure 2B).

Cellular studies using *S. cerevisiae* have provided evidence that Ku localizes to telomere foci near the nuclear periphery and that Ku80 co-localizes with the telomere binding protein Rap1 at these telomeric foci (73, 74). Similar experiments in *S. pombe* and mammals have not been possible due to the large abundance and uniform distribution of Ku throughout their nucleus (69); D.G. and D.J.C., unpublished results). Deletions of either Ku70 or Ku80 in *S. cerevisiae* were shown to alter the positioning of telomeric DNA in the yeast nucleus, and Ku, Rap1 and the Sir proteins (Sir2, 3 and 4) are released from the telomere in response to DNA damage (73, 74). It was suggested that *S. cerevisiae* telomeric DNA serves as a reservoir for Ku and other proteins that respond to physiological signals such as DNA damage (73). Additionally, it was reported

that the nuclear pore protein Mlp1 physically tethers Ku70 to the nuclear periphery, thus forming a link between the telomere and nuclear envelope in *S. cerevisiae* (75).

The *S. cerevisiae* telomerase RNA gene (TLC1) was originally isolated using a screen for genes that, when expressed in high amounts, would suppress telomeric silencing (39). TLC1 involvement in telomere silencing was generally unexpected and the nature of TLC1 RNAs role in telomere silencing remained unclear until recently. Some answers came in a recent report which demonstrated that overexpression of a 48nt stem-loop structure found within the TLC1 RNA (~1.3kb) was responsible for this suppression of telomere silencing and, additionally, caused telomere shortening (76). Furthermore, the phenotypes associated with TLC RNA overexpression were found to be similar to the phenotypic characteristics associated with yKu70 and yKu80 deficiencies. Overexpression of the 48nt stem-loop TLC RNA in cells deleted for Ku70 or Ku80 had no additional impact on telomere length suggesting they share the same functional pathway. This was contrasted by additional telomere shortening resulting from the deletion of several other telomere components, TEL1, TEL2, RAD50p or SIR4, in combination with overexpression of the 48nt stem-loop TLC RNA. However, it is important to recognize that a direct molecular interaction between the full-length Ku protein and TLC has yet to be demonstrated (76). Therefore, based on this rather tenuous genetic analysis, the authors concluded that Ku might be linked to the yeast telomerase RNA (76).

VII. Ku at the mammalian telomere.

The discovery that Ku plays a critical role at the yeast telomere, along with the fact that Ku and certain other components of the telomere are highly conserved from yeast to mammals, inspired studies into whether Ku played a similar role at the mammalian telomere. Using chromatin-immunoprecipitation (CHIP), Ku80 was found to localize to human and hamster telomeres as had been observed in yeast (77). It was also determined that the DNA-dependent protein kinase catalytic component (DNA-PKcs) was not required *in vivo* for the association of Ku80 with telomeric repeats (77). Additional CHIP *in vivo* cross-linking studies confirmed that both Ku80 and Ku70 are localized to the mammalian telomere (60). As with the yeast studies, these CHIP findings indicate a direct physical link between Ku and the telomere in mammalian cells but do not determine whether Ku binds directly to telomeric DNA. In regard to Ku binding directly to telomeric DNA, it was reported that purified Ku protein is capable of binding to mammalian telomeric DNA ends in vitro (78). However, as previously stated, it is well know that the Ku protein binds non-specifically to DNA double-strand ends, so the biological significance of this result is unclear. Nonetheless, it is clear from these studies that some portion of Ku is in close proximity to the telomere.

Additionally, it has been demonstrated that Ku plays a biological role at the mammalian telomere by the accumulation of telomere fusions in mouse cell lines deficient for Ku (10). However,

high background levels of telomere fusions are often observed in transformed mouse cell lines derived from wild type primary cells (DG and DJC, unpublished results). The telomere fusions observed in these transformed cell lines may result from spontaneous genomic alterations associated with the transformation process. Thus genomic alterations associated with transformed cells may cause the telomere fusions and be unrelated in this case to a Ku deficiency. But a confirmation of this finding recently came from several groups reporting the accumulation of telomere fusions in various primary mouse cells deficient for either Ku70 or Ku80 deficiencies (8, 60, 61), confirming that Ku is critical for telomere capping.

The mammalian telomere binding protein TRF1 localizes at telomeres by binding specifically to telomeric DNA and plays a role in telomere length regulation (79, 80). Recently it was reported that Ku forms a high affinity protein/protein interaction with TRF1 (8). The Ku and TRF1 complex is a specific high affinity interaction (K_D of 0.4 x 10^{-9}M), as determined in vitro by Biacore analysis, co-immunoprecipitation and, Far Western analysis. The Ku/TRF1 complex exists in human cells as determined by co-immunoprecipitation experiments. An electrophoretic mobility shift assay (EMSA), using micro-circular DNA substrates containing internal telomeric repeats, showed that Ku does not bind mammalian telomeric repeats site-specifically, but localizes to telomeric repeats via its high affinity interaction with TRF1 (8). Thus, Ku protein function at the telomere is different than during joining non-homologous DNA double-stranded breaks. This suggests that once Ku forms a complex with TRF1, Ku specific DNA repair domains required to tether broken DNA ends and recruit other DNA repair proteins may be obscured to prevent DNA end joining activity at the telomere (29).

TRF2, a telomere repeat binding protein that localizes to t-loop junctions (Figure 3C; **(55)**, can interact with an undetermined affinity to a truncated version of Ku70 (consisting of amino acids 200 to 385) **(81)**. However, since full length Ku70 and TRF2 fail to form a complex (81), the biological significance of this finding is unclear. Regardless, if not DNA mediated, a Ku70/TRF2 complex might form transiently at the telomere dependent on the conformation or exposure of a specific domain within Ku70.

Although Ku has been localized to the mammalian telomere and plays a critical role in capping, it was still uncertain whether Ku also had a role in telomere length maintenance. Two independent groups using quantitative fluorescence in situ hybridization (qFISH) have published different results on this topic. Initially, one group reported that telomeric DNA remains at wild type lengths in Ku80 deficient mouse embryonic fibroblasts (MEFs) (61). Then recently it was reported that telomere length is reduced significantly, about 40%, from wild type telomere length in both Ku70 and Ku80 deficient cells from a variety of different cell types at various developmental stages (60). These conflicting results are likely due to technical differences in performing qFISH. Clarification of whether Ku functions in telomere length maintenance in mammals is absolutely essential to understand the basic role of Ku at the telomere.

In contrast to the action of yeast Ku in response to DNA damaging agents (73), human Ku does not dissociate from the telomere upon exposure to ionizing radiation and the radio-mimetic drug phleomycin (60). Since Ku is distributed throughout the mammalian nucleus, apparently its recruitment from the telomere to sites of DNA repair is not necessary (73).

VIII. Telomere shortening and cellular senescence.

Before our final discussion concerning the possible role of Ku in cellular senescence and aging, we will briefly review the role of the telomere in these processes. A minimal telomeric DNA length is required for chromosome stability and cellular viability, with failure to maintain telomere length leading to replicative senescence in yeast and mammalian cells (49). Telomeres in somatic cells shorten by 50-200 bp per cell division in the absence of telomerase activity and telomere length has been correlated with the proliferative capacity of normal cultured human cells (82). In contrast to most somatic cells, telomeres are maintained by an active telomerase in germline and most immortalized cells (83). Accordingly, activation of telomerase is a characteristic of most established cell lines and tumors (84, 85). One model accounting for the involvement of telomeres in the proliferative capacity of normal human cells proposes that telomere shortening during successive cell division serves as a counting mechanism that prevents unlimited proliferation of human somatic tissues (59). Programmed telomere shortening in normal human cells has been viewed as a tumor suppressor mechanism limiting the growth potential of certain cells that may acquire genetic defects (86). Intriguingly, activation of telomerase, via the ectopic expression of telomerase component TERT, allowed certain human primary and virus-transformed cell lines to bypass senescence and crises and continue proliferation (58, 87). However, although telomeres seem to play a critical role in the replicative capacity of certain human cells cultured in the laboratory, whether or not telomere maintenance is a critical factor in the aging of humans has yet to be established and is an area of active debate.

IX. Premature replicative senescence in mice deficient for Ku.

Embryonic fibroblasts taken from either Ku70 or Ku80 knockout mice revealed a pleotropic phenotype, which includes premature cellular senescence. Telomere maintenance appears to be a critical event controlling the ability of cells to continue proliferating in cell culture (58, 87). Therefore, factors that control telomere maintenance are potential candidates for components that block proliferation and lead to cellular senescence. Because of the link between the replicative capacity and telomere length, the reduction in the relicative capacity of Ku-deficient MEFs may simply be due to loss of telomere length regulation (14, 88). Furthermore, the accumulation of DNA damage during aging has been suggested by many as a possible cause of aging and cellular senescence (89). In fact, during aging and cellular senescence mutations accumulate in genomic DNA. Therefore, loss of DNA repair capacity might increase the accumulation of DNA damage and play an important role in cellular senescence and aging. In this regard, both Ku70 and Ku80

show significant decreases in steady state levels during replicative senescence of human fibroblasts (90). The actual mechanistic operation of Ku during the process of cellular and organismal aging will be important to determine to further our understanding of the aging process.

X. Conclusion

The telomere is likely a dynamic structure, changing conformations depending on the stage of development or cell cycle, with proper maintenance of telomere capping required at all times to prevent telomere fusions. Therefore, it seems paradoxical that the DNA repair enzymes involved in NHEJ are localized to the telomere. It is conceivable that DNA repair proteins were recruited to the telomere at some time during or after the evolution of linear genomes for a divergent telomere specific function.

At the mammalian telomere, the 3' telomeric DNA single stranded overhang can be buried in the t-loop form with telomeric proteins bound to stabilize and conceal the t-loop junction (Figure 3C; (55). However, during telomeric DNA replication, the 3' end must be single stranded to allow telomerase access to anneal to the telomerase RNA template for telomeric DNA replication (Figure 2C and Figure 3D). Though no evidence currently exists, Ku could load onto telomeric DNA utilizing its non-specific end binding activity at double strand DNA junctions (Figure 2C and Figure 3C). Though even as the 3' telomeric DNA overhang is unwound it is likely associated with specific telomere associated proteins and telomerase (Figure 2C and Figure 3D; (91)). In yeast, the telomere is apparently not exposed as naked double-strand DNA but has been shown to be associated with specific telomeric single and double-stranded DNA binding proteins and telomerase (Figure 2C; (92). Given the telomeric DNA end is capped or physically coated with telomere associated proteins, Ku might not be physically capable of loading directly onto the telomeric DNA termini. Since Ku and TRF1 form a high affinity complex in the human cell and essentially all TRF1 localizes to the telomere, these results give a possible mechanism for Ku localization to the capped telomere via complex formation with TRF1 (Figure 3A). Yeast Ku protein might also localize to the telomere via a protein-protein interaction (Figure 2B).

Ku proteins functional mode likely depends on its nuclear microenvironment. The nuclear microenvironment dependent on potential protein, DNA and/or even RNA contacts associated with Ku. In summary, as Ku has many different functional capabilities and interacting partners, it is important to clearly assess findings in relation to Ku proteins role at the telomere and not be biased by preconceived ideas from its possible roles in other cellular processes.

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Legends

Figure 1. Ku has been observed to form three different types of intermolecular contacts; 1) Sequence-independent DNA end binding 2) sequence-dependent DNA/RNA binding 3) Protein-protein associations and these interactions are likely important for its functional role in the cell.

Figure 2. Model of Ku at the yeast telomere. This schematic does not attempt to show all known telomere components at the yeast telomere but rather centers on proteins related to Ku proteins function at the telomere. Telomeric DNA is shown as blue lines. A) Rap1 and Sir4 may form a complex at the telomere. B) Model suggesting that yKu might localize to the telomere via an interaction with an unidentified protein (denoted by a question mark) that binds directly to telomeric DNA. Ku has been reported to interact with Sir4 (48, 71). Ku localization to the telomere is not bridged directly through Sir4 (71). C) Ku may localize to the telomere by binding directly to the double strand DNA end, although direct telomeric DNA end binding of Ku might not be possible due to capping of the telomere end by telomerase, Cdc13, Stn, telomerase and other putative telomere end binding proteins (54, 92-94).

Figure 3. Model of Ku at the mammalian telomere. As with the yeast telomere model in Figure 2, this schematic focuses on telomere components that may interact with Ku at the telomere and does not attempt to show all known mammalian telomere components. Blue lines represent telomeric DNA. A) TRF1 interaction with several telomere proteins; Ku, Tin2 and Tankyrase (8, 95, 96). B) Ku might localize to the telomere via an unidentified interaction with a telomere component (putative unidentified protein denoted by question mark) and may form a complex with telomerase. C) T-loop and replicative conformations of the mammalian telomere (55). Ku could load onto telomeric DNA directly via its non-specific end binding activity at a double strand DNA junction either in the t-loop or replicative telomere form. TRF2 is shown bound to the t-loop junction. Pot1 is shown bound to the G-rich single-strand telomeric DNA (91). Telomerase must bind to the 3' telomeric DNA during the telomeric DNA replication.

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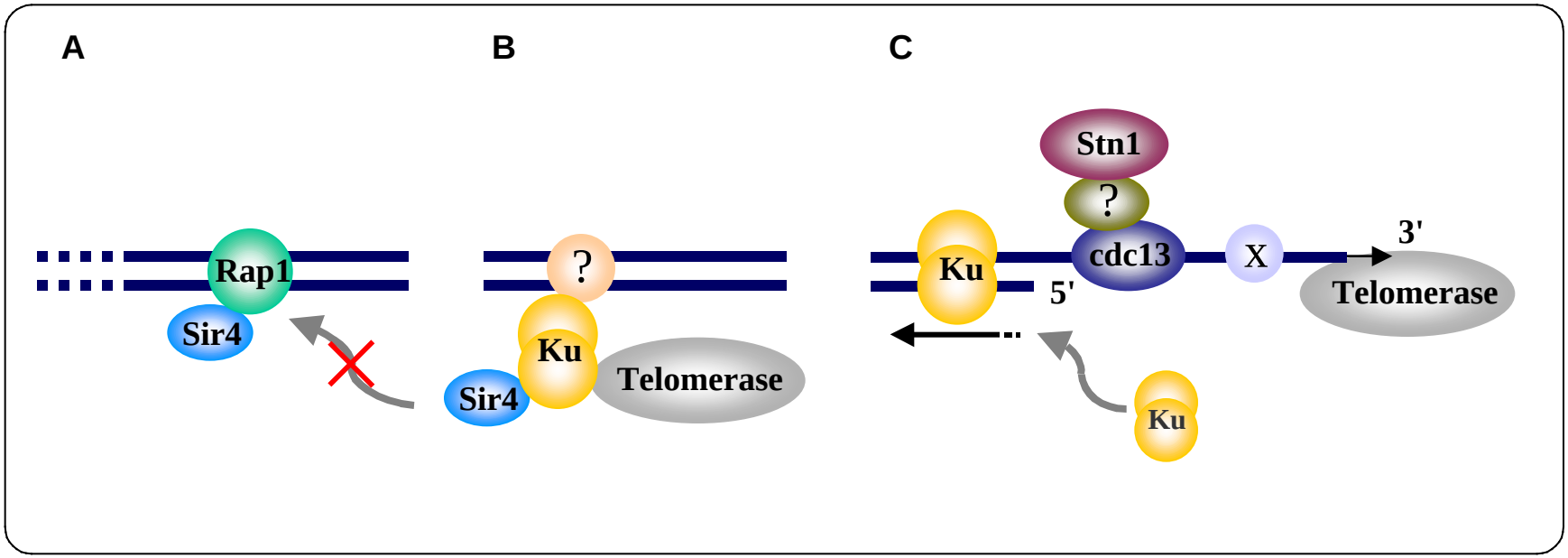
A
B
C
Rap1
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Telomerase
Stn1
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cdc13
X
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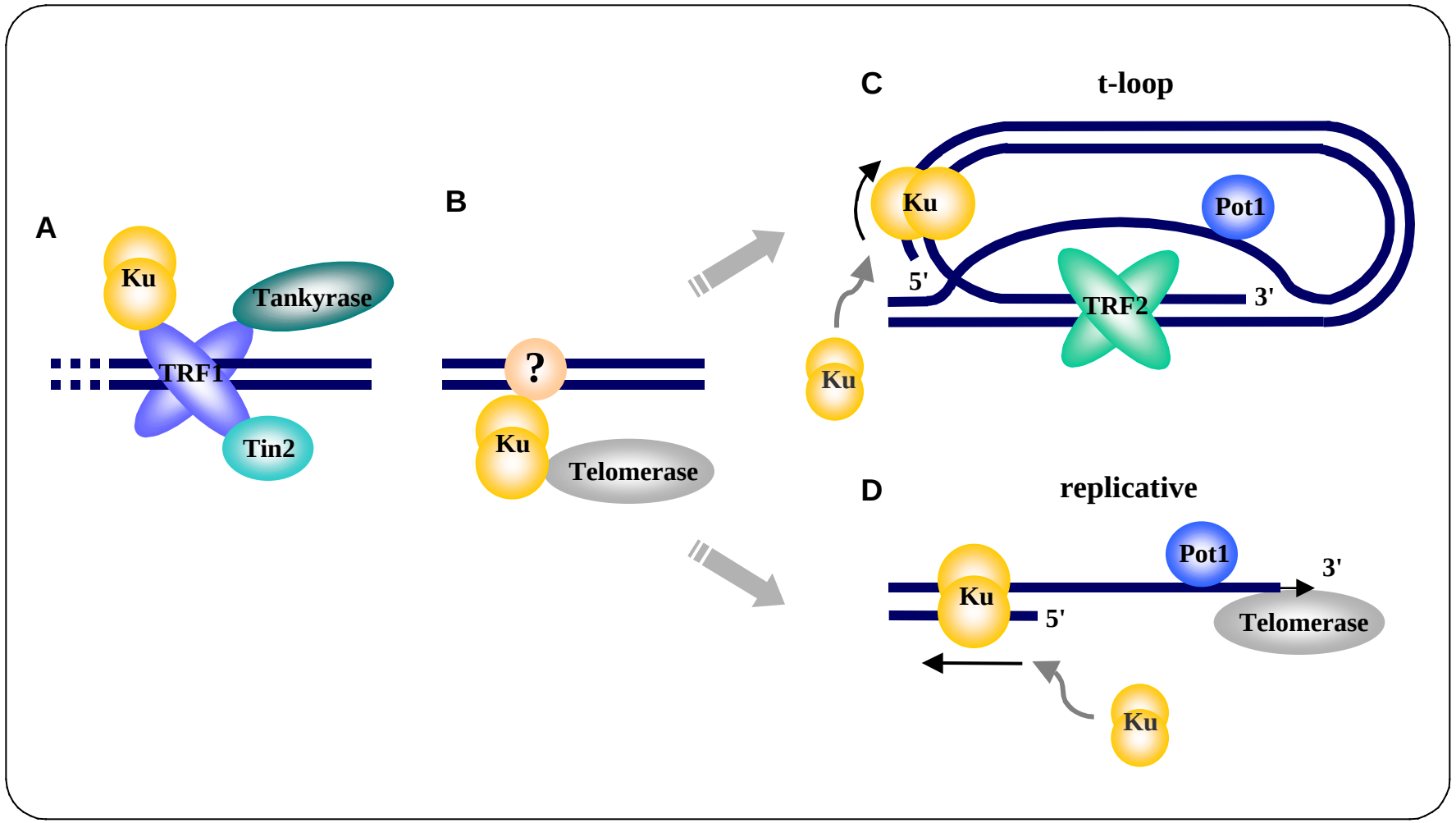
Telomerase
5'
Ku

Figure 1


A
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Telomerase
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Figure 2

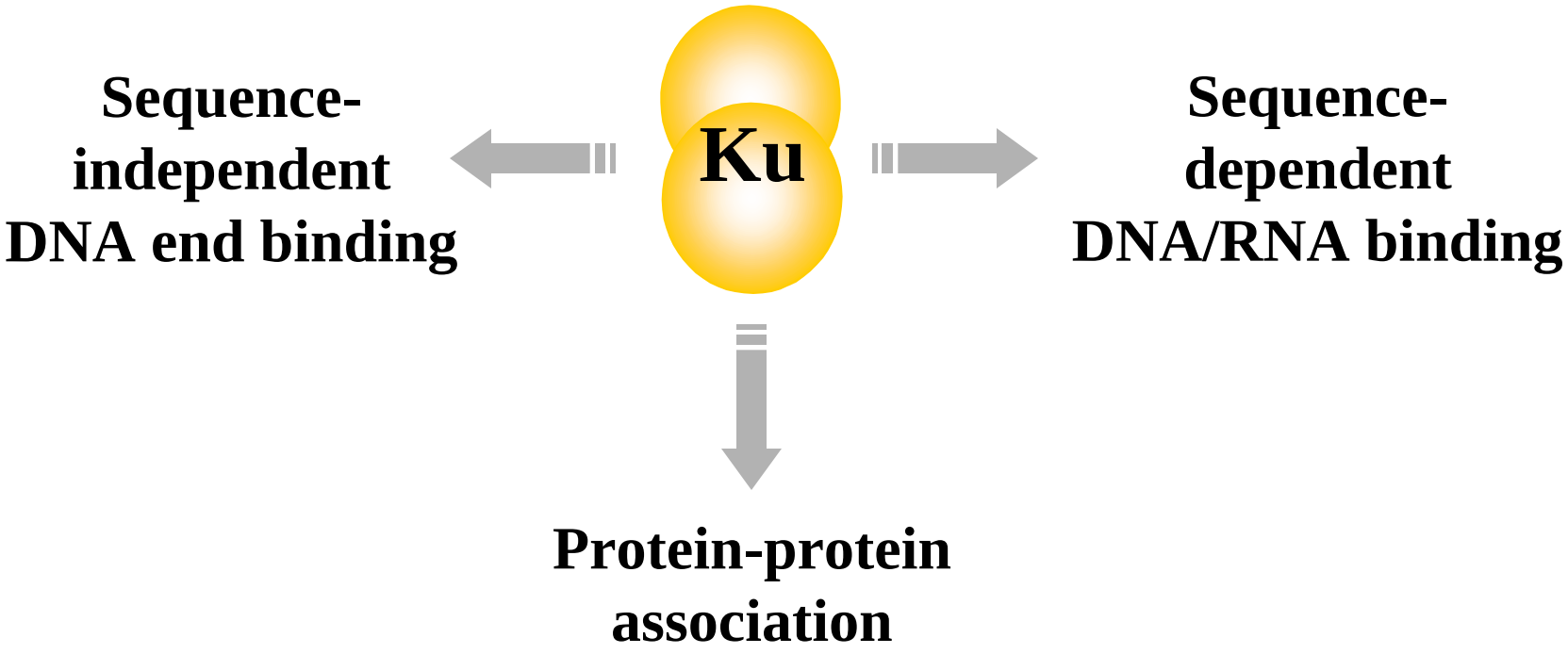
Sequence-
independent
DNA end binding
Ku
Sequence-
dependent
DNA/RNA binding
Protein-protein
association

Figure 3